SOME ASPECTS OF AMITOSIS IN SYNCHYTRIUM[1]

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(WITH PLATES III AND IV)

Previous papers on the cytology of Synchytrium have announced very striking peculiarities in the nuclear behavior of this interesting fungus. The idiosyncrasies, only a portion of which have yet been described, are so abundant at a certain period of the life-cycle of the plant that it is very difficult to consider any one set of phenomena without quickly becoming involved in all the rest, either because of the occurrence of different types of structures in the same coenocytic cyst, or because of transitional forms apparently connecting diverse structures. While no final interpretation of any one series of nuclear transformations can be made until it has been brought into relation with the whole life-history, it is apparent that it is out of the question to work out all of the peculiarities at once. The present paper is an attempt to isolate and describe one of the most conspicuous groups of nuclear phenomena. Further correlation of this with other manifestations of nuclear activity will be undertaken in later papers.

As in the preparation of a former paper on Synchytrium (GRIGGS **7**), the writer is under very great obligations to his friend, Professor F. L. STEVENS, for the information which aroused his interest in the problem and for criticism of the results. This obligation is increased by the fact that Dr. STEVENS also supplied the material from which the slides were made. The present paper deals entirely with one species, *Synchytrium decipiens* Farlow. The drawings have all been taken from preparations stained with Heidenhain's iron alum hematoxylin. The triple stain has also been used.

In the cytology of this plant there is no more striking feature than the variation in the size of the nuclei. In the same cyst nuclei are frequently found ranging all the way from 8 or 10 μ down to 1 μ in diameter, as was first reported by STEVENS (**12**, *fig. 2*). Very often the small nuclei are bunched together, either in a close morula-like

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cluster (*fig. 33*) or in a looser group (*fig. 18*). The origin and fate of these small nuclei is the subject of the present paper. Although such variations in the size of the nuclei are sometimes found in almost any stage of the period of nuclear division, they are most conspicuous immediately after the division of the primary nucleus and continue prominent until there are 200–300 nuclei in the cyst. It is at this same stage that the other peculiarities in the cytology are most pronounced. While this period of irregularities is not sharply marked off from the succeeding phases of the life-history, yet as the nuclei become more and more numerous there seems to be a tendency for them to settle down, so to speak, and to conform more nearly to the usual habits of dividing nuclei in growing tissue.

The isolation of these groups suggests that their constituent nuclei have a common origin. Because of the absence of any pairing, and because of their great differences in size, one is inclined to suspect that they have been derived by some process other than mitosis. Since mitosis in this plant is always simultaneous, involving all the nuclei in a cyst, the differences could not be due to the failure of some nuclei to divide, while their neighbors became smaller and smaller by repeated division. They might of course be due to some process of mitosis in which the products were unequal, as in the reduction division of an animal egg. But all the mitoses observed gave rise to equal daughter nuclei. Further, mitoses in cysts of this age are uncommon. This led STEVENS (**12**) to suggest the possibility of an amitotic origin for the nuclei of this stage.

There are several processes of direct nuclear division in Synchytrium. Two of these are quite different from the commonly observed division by an amoeboid constriction of the parent nucleus. While they may be considered under the general term amitosis, which has come to include several forms of non-mitotic division, they require distinctive terms for their designation. Indeed, there is considerable need for a classification of the different forms of direct division, especially in view of the increased importance amitosis is likely to assume in future cytological discussion. The first process, which consists of a budding-out of a small nucleus from a larger, may be designated *nuclear gemmation.* The second differs from ordinary amitosis in that the nucleus loses its membrane and vacuole of karyo-

lymph before the division, which is a multiple fragmentation. This form of division I shall term *heteroschizis* (ἕτερος, different, and σχίζειν, to split).

NUCLEAR GEMMATION

In nuclear gemmation, as is usual in amitosis, the division of the chromatin is not nearly so frequently observed as the separation of the two nuclei. In the resting nuclei of Synchytrium the whole of the chromatin content is usually concentrated in a single globular karyosome (nucleolus). At the beginning of nuclear gemmation the margin of this karyosome becomes crenate, and rounded lobes develop, which separate from it and become smaller independent karyosomes (*figs. 4–8*). Sometimes only one daughter karyosome migrates from the parent at a time (*fig. 6*); sometimes the parent undergoes a process of bipartition resulting in equal daughter karyosomes (*fig. 7*); or sometimes several form at once, in which case the whole karyosome breaks up (*fig. 4*). *Fig. 8* shows a very large nucleus where the daughter karyosomes were unusually numerous. They were not free in the nucleus, as appears from the drawing, but all of them were lying against the nuclear membrane, only one hemisphere of which is represented.

After the separation of the small karyosomes is complete, *they migrate through the nuclear membrane.* This process is probably rather gradual, since all stages are easy to observe: *figs. 8, 19* show them lying loosely against the membrane; in *figs. 9, 20* they are pressed against it; in *figs. 8, 21* they have begun to pass through; *fig. 21* shows one lying almost exactly half-way through the membrane; *figs. 10, 11* show karyosomes which have passed through, but still lie close against, the membrane.

As soon as the passage is completed, a vacuole, similar to the cavity of the parent nucleus, appears around the migrating karyosome. This is quickly surrounded by a membrane, extending out from the wall of the parent nucleus into the cytoplasm next the vacuole (*fig. 10*). This process can be observed satisfactorily only when the daughter nucleus is of considerable size, because of the delicacy of the membranes of the smaller nuclei. When the membrane is complete the new nucleus moves away from the parent and becomes an independent

small nucleus free in the cytoplasm. The stages in this process are also easy to follow: *figs. 3, 9, 11* show cases where the karyosome is still in contact with the membrane of the parent; *figs. 6, 12, 13, 18* cases where the karyosome has separated from the parent, but the membranes remain in contact; *figs. 3, 5, 9, 14* cases where the two nuclei have separated, but still lie close together.

Division by nuclear gemmation occurs also in the spirem stage (*figs. 15–17*). In this case the division of the chromatin takes place at the time of spirem formation and cannot be definitely connected with nuclear division, but the manner of the separation of the daughter nuclei is the same as that already described. *Figs. 1, 2* show groups of small nuclei from a cyst where all the large nuclei (*fig. 16*) are in spirem.

In cysts where the nuclei are numerous and evenly scattered through the cytoplasm it can be seen that the peripheral nuclei divide much earlier than the central ones. Groups of small nuclei are always found at the periphery before the large nuclei in the center are much divided. Thus a lateral section of a cyst (*fig. 1*) shows only uniform groups of small nuclei, while the central sections show numerous large nuclei, of which *fig. 16* is an example.

Nuclear gemmation may take place at very different rates in different cysts. In the cyst from which *figs. 11, 12, 14* were taken, the few small nuclei present are scattered singly through the cytoplasm. In this case the appearances indicate a slow and orderly formation of small nuclei. In other cysts the chromatin seems to be extruded with almost explosive violence (*figs. 24–26*, cf. also GLASER **6**). In these cases a large proportion of the migrating chromatin never forms nuclei but degenerates in the cytoplasm. Some members of almost every large group are imperfect and disintegrate, forming in their last stages deeply staining spots in the cytoplasm. Such disintegration is seldom seen in cysts where the small nuclei give evidence of more gradual formation. It is more pronounced in younger cysts where there are only a few parent nuclei, than in later stages where they are numerous.

The deeply staining granules on the nuclear membrane vary from karyosomes half the size of the mother karyosome to microsomes similar to those usually found in the nuclear membrane in both

animal and plant cells (*figs. 7, 18, 21, 22*). No optical distinction can be drawn between these extremes. The very smallest granules, however, do not form small nuclei but may function in metabolism. In mitosis and in the degeneration of the large nuclei (*fig. 22*) they are cast aside with the old nuclear membrane and lost in the cytoplasm. But no distinction can be drawn between these granules and those which form small nuclei, for some of the latter are excessively minute. Besides these, there are yet other granules on the nuclear membrane from which conspicuous radiations proceed into the cytoplasm as from centrosomes (*fig. 39*). The discussion of these bodies involves other questions than those considered in the present paper and cannot be undertaken here. Another complicating factor is the frequent presence of asters near nuclei which are giving off gemmae. I have avoided using such cases for the figures of the present paper, but in many instances nuclei adjoining those drawn had conspicuous asters, and it would be possible to duplicate most of the drawings herewith given from nuclei showing asters. But though the centrosome problem, one aspect of which was touched in a former paper (GRIGGS **7**), is very puzzling and far from solution, my belief is that it is independent of the phenomena discussed in the present paper.

HETEROSCHIZIS

The second process of amitosis is a multiple division or fragmentation of the nucleus, which occurs for the most part at later stages than nuclear gemmation, but is sometimes found in young cysts (*fig. 33*) and rarely also even in segmented cysts (*fig. 34*). Nuclei derived by heteroschizis are at once distinguished from those due to nuclear gemmation, because they form not a loose group but a close morula-like cluster, as figured by STEVENS (**12**, *fig. 3f*). As in nuclear gemmation, all the stages in their formation are easy to observe and may be found in a single cyst. But while the new nuclei are formed one at a time in that process, here they originate simultaneously by the fragmentation of the mother nucleus. The first indication of division consists in the loss of the nuclear membrane and the vacuole of nuclear sap, leaving the karyosome lying naked in the cytoplasm, like the chromosomes in the metaphase of mitosis (*fig. 27*). The karyosome then apparently enlarges to nearly double its former size (*fig.*

28). This statement is based on the fact that the naked karyosomes are, in the cases observed, larger than those of any other nuclei in the same cysts, and that the resultant clusters of small nuclei are greater in mass than any single nucleus in the cyst at this stage. The variation in the size of the nuclei, however, is so great in other stages that it is not impossible that these may have been larger nuclei in the beginning. Lobes (*fig. 29*) now appear on the margin of the karyosome, each of which rounds off and becomes the karyosome of a small nucleus. When these karyosomes have separated, vacuoles of nuclear sap appear around them; surrounding membranes are next formed in the meshes of the cytoreticulum bounding the cavities, thus completing the process. The membranes, however, do not appear simultaneously around all the nuclei of a cluster. There is usually sufficient difference to allow some observation of the process of membrane formation. The vacuoles which become the nuclear cavities are at first indistinguishable from those between the meshes of the cytoreticulum which are filled with cell sap, but they are gradually surrounded by membranes which are apparently precipitated from the cytoplasm next the cavity. Neither in heteroschizis nor in nuclear gemmation is there evidence of any connection of the centrosomes with membrane formation such as occurs in the reconstruction of the nucleus after mitosis (KUSANO **8**, GRIGGS **7**).

Besides the two sorts of amitosis just described, a third method has been observed a few times. In this process, which has been seen only when the nuclei were in spirem, the nucleus becomes strongly lobed; each lobe contains a portion of the original unchanged spirem; the lobes become more pronounced and are cut apart by continued constriction. There may be only two lobes, as in ordinary amitosis, or there may be several, as in heteroschizis. Although even a single nucleus of this kind (*fig. 40*) would seem to indicate the general nature of the process, there is much concerning it which is doubtful, and its occurrence is rare in my slides. I refrain, therefore, from more than mention of the matter at this time.

LATER HISTORY OF THE SMALL NUCLEI

If amitosis leads to degeneration and death, as has been held almost universally until recently, we should expect to find a large

percentage of degenerating small nuclei in every cyst where they occur, either during the period of their formation or later. After nuclear gemmation, however, degeneration of chromatin is relatively small in amount and is almost altogether confined to masses which never organize nuclei. It occurs not in the later portion of the period of gemmation but only during the early portion, when there are few large nuclei in the cysts. In clusters due to heteroschizis, degeneration may also occur at later stages, but is infrequent at any time. If, on the other hand, these amitoses are due to pathological conditions affecting the whole parasite, we should expect to find a large number of dying cysts. Fully three-fourths of all the few-nucleate cysts give evidence of amitosis. Of the remainder only a small number show mitoses at this stage. This hypothesis would therefore require that three-fourths of the cysts should degenerate sooner or later. But no such thing occurs. Degenerating cysts are seldom found, and the degeneration gives no indication of being connected with earlier amitoses.

The clusters of small nuclei arising from heteroschizis tend to remain close together, and when mitosis is resumed they may form a cluster of small spindles. *Fig. 35* shows such a cluster between prophase and metaphase, in which the remains of the nuclear membranes are still evident. Below them is the solitary spindle of a large nucleus, of which there are 40–50 in the cyst. *Fig. 36* shows three objects from another cyst assembled in one drawing. At *a* is a similar cluster of small spindles; at *b* is one of the solitary spindles of the larger nuclei, all of which in this cyst are in a later phase than the clusters; at *c* is a deeply staining mass which has the appearance of a cluster similar to *a* degenerating. *Figs. 37, 38* show similar clusters in anaphase. Although the spindle fibers in *fig. 38* are distorted so as to give somewhat the appearance of a pathological multipolar spindle, some of the spindles are perfectly normal. The spindles of *fig. 37* resemble closely the solitary spindles of the cyst and are typical examples of the peculiar anaphases of this genus.

The groups of small nuclei arising by nuclear gemmation scatter quickly, so that there is no means of connecting them with the mitoses which occur later. They have the usual relations, however, to the cytoplasm and appear normal in all microscopic characters. When

not too small they bud off other small nuclei in the same manner (*fig. 18*). This process usually continues till all the nuclei in the cyst are approximately equal in size (*figs. 1, 2*). Sometimes all the daughter nuclei given off are so much smaller than the parent that the mother karyosome is never divided up among the daughters, but remains behind full size, after giving up its chromatin, like the nucleolus in the prophases of mitosis. In this case the *large nuclei* degenerate and leave the small ones as the functional nuclei of the cyst. *Fig. 20* shows the first indication of this in the vacuolate karyosome of the parent nucleus. In *fig. 21* all the chromatin has migrated from the old karyosome but some of the small karyosomes still remain inside the nuclear membrane. The larger of these are about the same size as the numerous small nuclei of the cyst. *Fig. 22* shows another large nucleus from the same cyst, which is entirely bereft of chromatin. *Fig. 23* is the last stage of the process; here the old nuclear membrane has disappeared and the faintly staining old karyosome (nucleolus) lies naked in the cytoplasm. Beside it is shown one of the functional nuclei. We are therefore led to the conclusion that the nuclei derived by these processes of amitosis are normal, and that they with their descendants become the functional nuclei of later stages, capable of perpetuating the species.

GENERAL CONSIDERATIONS

Although the processes by which these nuclei are derived are novel, the formation of normal tissue by amitosis is by no means without parallel. C. M. CHILD (**1–5**) has recently shown that amitosis is a frequent occurrence in regenerating organs, embryos, and in some adult animals. He records instances from most of the great animal phyla, including coelenterates, flat worms, trematodes, cestodes, insects, amphioxus, fishes, amphibia, and birds. In these cases, contrary to what would be expected, there seems to be no especial distinction between the soma and the germ plasm as to the origin of the nuclei. In Moniezia, a tapeworm infesting sheep, which CHILD has worked out most fully, the germ plasm is almost exclusively derived by amitosis and the spermatogonia may even undergo a sort of amitotic reduction by which sperms are formed without ever having passed through mitosis. In general, amitosis is most common in

regions of excessively rapid growth, where the nuclei are small and have scant cytoplasm, while the larger nuclei, better supplied with cytoplasm, divide by mitosis. This leads CHILD to conclude (**5**, p. 292): "In short I am inclined to believe that amitosis is associated with conditions where the demand for material or perhaps for some particular substances exceeds the supply." The behavior of the nuclei of Synchytrium is distinctly opposed to the generalization of this hypothesis, for in Synchytrium amitosis is most marked when the nuclei are largest and the ratio of nuclei to cytoplasm is at a minimum. While a condition of "hunger" may very well be assumed to exist in the cells of a rapidly regenerating organ or in a growing embryo, it cannot be ascribed to a dividing cyst of Synchytrium, because growth is very slight after the division of the primary nucleus, while the supply of nutriment from the host is presumably as great as before.

But in all of these cases the conditions of growth demand an excessively rapid multiplication of nuclei, and indicate that the process of nuclear reproduction is pressed on so rapidly as to give no opportunity for the rhythmic pause occasioned by mitosis. If the stimuli to growth and reproduction are independent, as many observations indicate, we may suppose that when the stimulus to division becomes excessive the nucleus divides directly, without waiting for the long preliminary pause necessary in mitosis. If the stimulus were but slightly stronger than in mitosis, a slow and orderly division of the chromatin would result, and the daughter nuclei would be mostly perfect; but if the stimulus became greater the process would be accelerated, until finally the nucleus would explode and a large proportion of the chromatin would never succeed in forming new nuclei at all. This is exactly in line with CHILD'S view that amitosis is an orthodromic process which ". . . . pushed to the extreme must always result in the total destruction of the original substances," so that "it is not strange that degeneration frequently follows amitosis, but there is no reason for supposing that it must always follow, and the facts prove that it does not." While this hypothesis may not cover cases of pathological amitosis, which superficially would seem to accord with the hunger hypothesis, it would afford a basis for associating the non-mitotic divisions in Synchytrium with those in regenerating members, embryos, and other rapidly growing tissues.

Knowledge that in certain instances the reproductive cells of a species are independent of mitosis for their origin must affect current theories of heredity, which, since the renaissance of MENDEL'S law, have leaned very heavily on the individuality of the chromosomes and their separation in the reduction division. CHILD rejects the chromosome theory in any universal application. He believes (p. 290) that "these processes appear to consist essentially in the production of new nuclear material like that already present and without the periodical recurrence of metamorphosis. The act of division is very probably a mere incident of the increasing volume of substance." Accordingly he is inclined to doubt the constancy of the chromosome number in the tapeworm, although he feels that the facts are too difficult of determination to admit of certainty. In Synchytrium, likewise, the minuteness of the nuclei makes determination of the chromosome number so difficult that one hesitates to dogmatize. But in all the many cases in which the chromosomes could be counted on the spindle the number seemed to be constantly four (cf. *fig. 36b*). The same number was given provisionally by STEVENS (**13**) in his first paper and is shown by the drawings of his second paper (**12**). This matter may, however, be left for consideration later, after the mitoses have been worked out in detail. But whether the chromosome number is found to be constant or variable, it is obvious that our theories of heredity will require considerable revision.

SUMMARY OF RESULTS

The numerous peculiarities in the cytology of Synchytrium occur mostly in a somewhat definite *period of irregularities* immediately following the division of the primary nucleus.

In this stage direct division of the nucleus is more frequent than mitosis. This takes place by at least two processes:

1. *Nuclear gemmation.*—The karyosome of the parent nucleus gives off a small karyosome which migrates through the nuclear membrane, forms a vacuole and a membrane about itself, and becomes an independent small nucleus, the whole looking like a budding yeast plant. This process is repeated until the parent nucleus is converted into small nuclei, often forming a definite group.

2. *Heteroschizis.*—The membrane of the parent nucleus dissolves,

and the karyosome fragments into a number of pieces, each of which becomes a new nucleus, thus giving rise to a morula-like cluster of nuclei.

These nuclei at later stages undergo mitosis and their descendants form spores and become the nuclei of succeeding generations.

No variation in the number of chromosomes in any of the nuclei of the plant has been detected.

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EXPLANATION OF PLATES III AND IV

The figures were all made with a Spencer 1.5^{mm} immersion objective and compensating ocular 12, giving a magnification of 2130, excepting *fig. 1*, for

which ocular 2 (magnification 355) was used. They were reduced ⅓ in reproduction, canceling the enlargement due to the camera and rendering them the same size as they were seen in the microscope.

PLATE III

FIG. 1.—A lateral section of a cyst, showing numerous groups of small nuclei due to nuclear gemmation.

FIG. 2.—One of the groups shown in *fig. 1*.

FIGS. 3–6.—The breaking-up of the mother karyosome preparatory to the migration of the chromatin.

FIG. 7.—Bipartition of the mother karyosome to form equal daughters.

FIG. 8.—Nucleus with a large number of daughter karyosomes lying on the nuclear membrane, only one hemisphere of which is shown.

FIG. 9.—A nucleus with one of the daughter karyosomes pressed against the nuclear membrane; three small nuclei which have budded off from it near by.

FIG. 10.—Daughter karyosome constructing its nuclear cavity and membrane.

FIG. 11.—Daughter nucleus complete but still closely appressed to the membrane of its parent.

FIGS. 12, 13.—Karyosomes of daughter nuclei separated from the parents but their membranes still in contact.

FIG. 14.—Daughter nucleus separated from its parent but lying close by.

FIGS. 15–17.—Nuclear gemmation from the spirem stage. (*Fig. 16* is one of the large nuclei from the center of the cyst from which *figs. 1* and *2* were taken.)

FIGS. 18–20.—Resultant groups of small nuclei.

PLATE IV

FIGS. 21–23.—Stages in the degeneration of the parent nuclei.

FIGS. 24–26.—Nuclei from which chromatin has been thrown out in large quantities and is mostly degenerating without forming new nuclei.

FIG. 27.—Beginning of heteroschizis; nuclear membrane dissolving, karyosome slightly irregular.

FIG. 28.—Membrane and nuclear cavity lost, karyosome much enlarged. Same cyst as *fig. 27*.

FIG. 29.—Karyosome lobed. Same cyst as *figs. 27* and *28*.

FIGS. 30–32.—Karyosomes broken up, nuclear membranes appearing around the daughter karyosomes. (*Fig. 32* is from the same cyst as *figs. 27–29*.)

FIG. 33.—A very large cluster complete.

FIG. 34.—A single segment from a summer sorus whose nucleus has divided by heteroschizis.

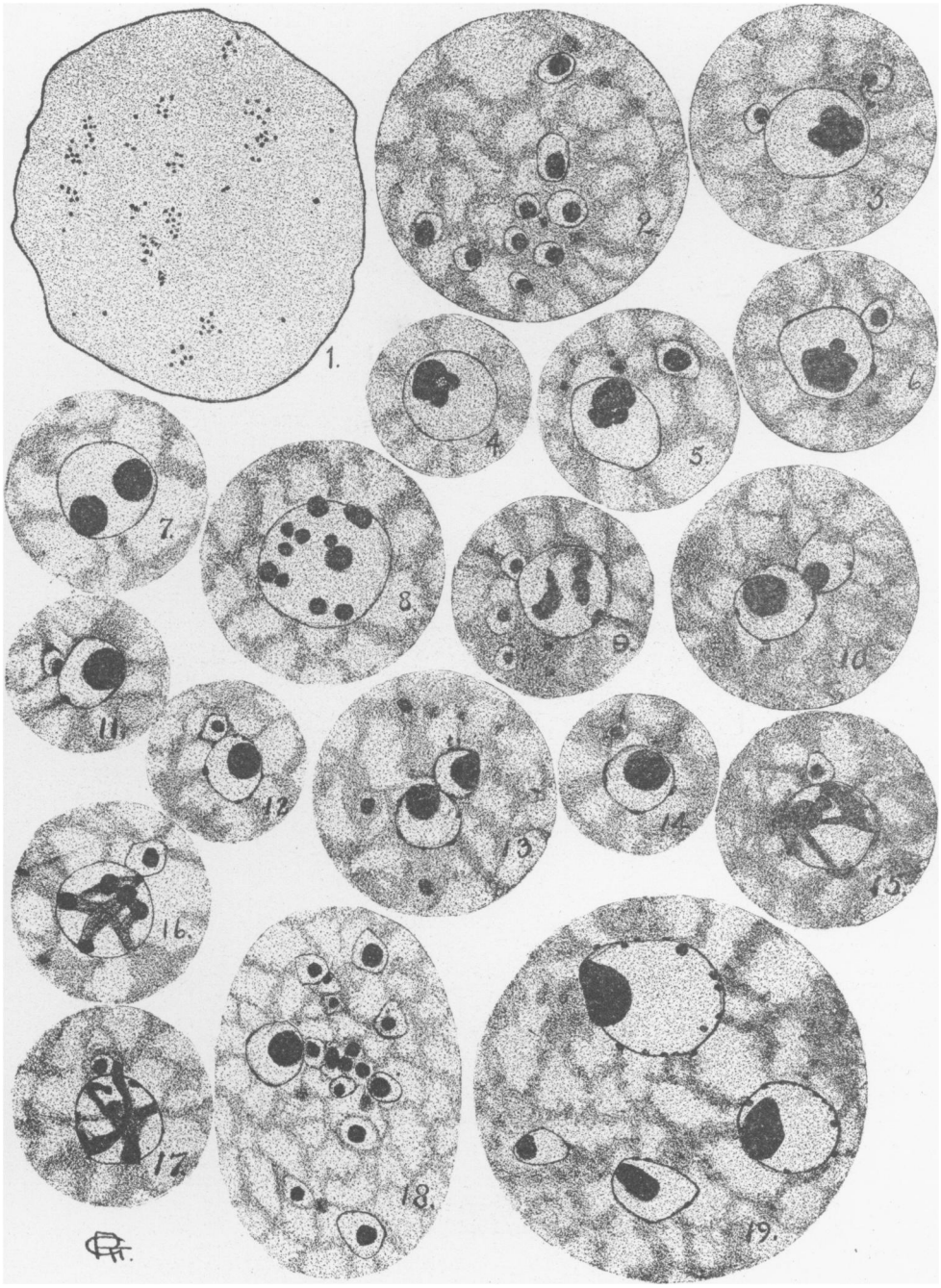
FIG. 35.—A cluster of spindles arising from the division of such a cluster as *fig. 33*, together with a single spindle of one of the solitary nuclei of the cyst.

FIG. 36.—*a*, a cluster of spindles similar to *fig. 35*; *b*, a solitary spindle from the same cyst; *c*, probably a cluster of spindles disintegrating.

FIGS. 37, 38.—Similar clusters of spindles in anaphase.

FIG. 39.—A nucleus with deeply staining granules on its membrane, from which radiations are given off into the cytoplasm.

FIG. 40.—A nucleus fragmenting by constriction.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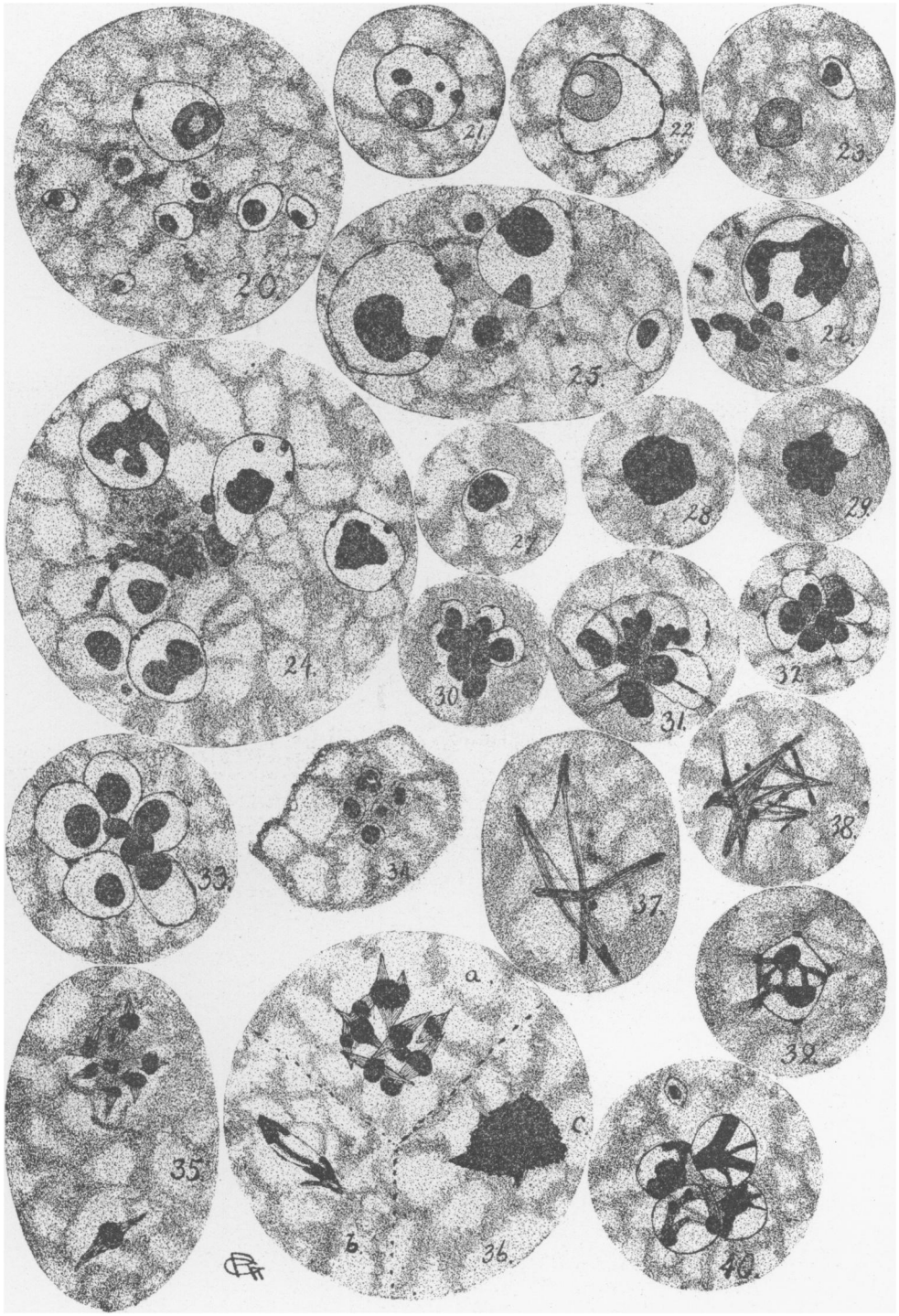
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